

WHAT IS CLAIMED IS:

1. An isolated and purified ATP diphosphohydrolase obtainable from bovine aorta characterized by the following physico-chemical properties:

5        -a catalytic unit of a molecular weight on denaturing polyacrylamide gel electrophoresis of about 78 KDa in its native form;

10        -a deglycosylated form of said catalytic unit of a molecular weight on SDS-PAGE of about 56 KDa; and characterized in that it comprises the amino acid sequences defined in SEQ. ID. NOS. 3 to 6.

2. An ATP diphosphohydrolase as defined in claim 1 further comprising the amino acid sequence defined in SEQ. ID. No.: 8 or a variant thereof.

15        3. An isolated and purified ATP diphosphohydrolase obtainable from pig pancreatic zymogen granules

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characterized by the following physico-chemical properties:

-a catalytic unit of a molecular weight on denaturing polyacrylamide gel electrophoresis of about  
5 54 KDa in its native form;

-a deglycosylated form of said catalytic unit of a molecular weight on SDS-PAGE of about 35 KDa; and characterized in that it comprises the amino acid sequence defined in SEQ. ID. NO.: 7.

10 4. A process for purifying <sup>thr</sup>an ATP-diphosphohydrolase enzyme <sub>of claim 3</sub> from a tissue capable to convert ATP to ADP and ADP to AMP which comprises:

a) obtaining a sub-cellular microsomal fraction from an homogenate of said tissue;

15 b) solubilizing said microsomal fraction in the presence of a non-ionic detergent;

c) centrifuging said solubilized microsomal fraction to obtain a supernatant containing said enzyme;

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d) submitting said supernatant to an ion-exchange chromatography to obtain a first enzyme eluate;

e) submitting said first eluate to an affinity column chromatography to obtain a second enzyme eluate;

5 and

f) submitting said second eluate to a separation step on a non-denaturing gel electrophoresis to recover said enzyme free of any contaminant, the presence of said contaminant being monitored by overstaining said gel in a silver nitrate dye or Coomassie Blue dye.

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5. A process according to claim 4 wherein said ion exchange chromatography is achieved on a column containing Diethylaminoethyl (DEAE).

6. A process according to claim 5 wherein said column is a DEAE agarose column.

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A 7. A process according to claim 4 ~~or 5~~ wherein said affinity column chromatography is achieved on an Affigel™ Blue column.

A 8. A process according to claim 4, ~~5, 6 or 7~~ wherein  
5 said non-ionic detergent is Triton X-100™.

A 9. A process according to claim 4, ~~5, 6, 7 or 8~~ wherein  
an aliquot of said enzyme is further submitted after  
step f) to a polyacrylamide gel electrophoresis under  
denaturing conditions to verify its homogeneity and to  
10 obtain its apparent molecular weight.

10. A process according to claim 9 wherein said enzyme  
is obtained from pig pancreatic zymogen granules and has  
an apparent molecular weight of 54 Kilodaltons.

11. A process according to claim 9 wherein said enzyme  
15 is obtained from bovine aortic intima layer and has an  
apparent molecular weight of about 78 Kilodaltons.

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12. A process according to claim 10 wherein, between steps e) and f), a step of deglycosylation is included, and whereby the apparent molecular weight is shifted from 54 to 35 KDa.

5 13. A process according to claim 11 herein, between steps e) and f), a step of deglycosylation is included, and whereby the apparent molecular weight is shifted from 78 to 56 KDa.

10 ~~14. The use of the ATP diphosphohydrolase of claim 1 or 2, for reducing platelet aggregation and thrombogenicity.~~

15 15. The use of an ATP diphosphohydrolase for reducing platelet aggregation and thrombogenicity, said ATP diphosphohydrolase having the amino acid sequence defined in SEQ. ID. NO.: 1, or a variant thereof, or a part thereof, said variant or part being capable of converting ATP to ADP and ADP to AMP.

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16. A composition for use in the reduction of platelet aggregation and thrombogenicity which comprises as an active ingredient the ATP diphosphohydrolase of claim 1 ~~or 2~~ or an ATP diphosphohydrolase which sequence is defined in SEQ. ID. NO.: 1, or a variant or a part thereof, which variant or part has an ATP diphosphohydrolase activity, in an acceptable pharmaceutical carrier.

17. A process for producing an ATP diphosphohydrolase which comprises the steps of:

- obtaining a host which comprises a nucleic acid encoding a protein having the amino acid sequence defined in SEQ. ID. NO.: 1, or a variant thereof, or a part thereof, said variant or part being capable of converting ATP to ADP and ADP to AMP;

- culturing said host in a culture medium supporting the growth of said host and the expression of said nucleic acid;

- recovering the ATP diphosphohydrolase from the culture medium or from said host; and
- purifying the ATP diphosphohydrolase.

5 18. A process as defined in claim 17, wherein said nucleic acid has a sequence defined in SEQ. ID. NO.: 2, a variant thereof or a part thereof, said variant or part being capable of producing an ATP diphosphohydrolase which converts ATP to ADP and ADP to AMD.

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